Human Peripheral Blood Lymphocyte Separation Solution

Please read the manual carefully before use.

Cat. No. FB102

Version No. Version 2.0

Storage: at 15℃-30℃ away from light for two years.

Description

Peripheral blood mononuclear cells (mainly lymphocytes) differ in size, morphology, and density from other cells. The density of red blood cells and granulocytes is relatively high, about 1.090 g/ml, the density of platelets is 1.030 ~ 1.035 g/ml, while the density of mononuclear cells is 1.075 ~ 1.090 g/ml. This product is a sterile, nearly isotonic, density of (1.077±0.001) g/ml (20℃) glucan and meglumin diatrizoate solution. When density gradient centrifugation of human anticoagulant blood is performed with this product, red blood cells and granulocytes with high density will sink in this separation solution, while the density of peripheral blood mononuclear cells (mainly lymphocytes) is slightly lower than the separation solution and will float in this separation solution, so lymphocytes with higher purity could be obtained. This product is ready-to-use. The isolated lymphocytes can be used for in vitro culture and immunological testing.

Component	FB102-02-V2
Human Peripheral Blood Lymphocyte Separation Solution	200 ml

Procedures

1. Bring the lymphocyte separation solution to room temperature before the experiment, and mix it upside down before opening the bottle cap. During the whole separation process, the temperature should be controlled between 15℃ to 25℃. Too high or too low temperature will affect the density of the separation solution, and then affect the separation effect.
2. Take fresh anticoagulated whole blood (EDTA, heparin or sodium citrate anticoagulant can be used) and dilute it with PBS or normal saline equilibrated to room temperature in equal volume.
3. Add the separation solution of the same volume as the undiluted whole blood into the centrifuge tube, and carefully add the diluted blood sample above the liquid level of the separation solution to keep the interface between the two liquid levels clear. At this point, the volume ratio of undiluted whole blood, PBS (or normal saline) and the separation solution is 1:1:1.
4. Centrifuge at 600-800×g for 20-30 minutes using horizontal rotor at room temperature, the speed is required to slowly rise and fall. The recommended separation condition for fresh peripheral blood within 4 hours after collection is centrifugation at 600×g for 20 minutes. The recommended separation condition for peripheral blood placed 4-8 hours after collection is centrifugation at 800×g for 30 minutes. Prolonged placement of blood samples will affect the separation effect.
5. After centrifugation, the solution in the centrifuge tube was divided into four layers from top to bottom, namely plasma layer, lymphocyte layer, separation solution layer and red blood cell layer. Among them, the lymphocyte layer is a thin and dense white film between the plasma layer and the separation solution layer. Carefully pipet the white film into the other centrifuge tube.
6. Dilute with 5-10 times the volume of PBS or normal saline and mix upside down. Centrifuge at 300×g for 10 minutes using horizontal rotor at room temperature.
7. Discard the supernatant and repeat step (6) once.
8. Resuspend lymphocytes with PBS or appropriate medium for later use.

Notes

- To maintain lymphocyte activity, separation should be performed as soon as possible after blood collection. Avoid refrigeration and freezing of blood during storage, handling and transportation.
- During blood collection and separation, perform aseptic operation to avoid microbial contamination.
- After opening, the lymphocyte separation solution should be stored at 4°C and used within 6 months to avoid density changes due to liquid volatilization that affect the separation effect.

For research use only, not for clinical diagnosis.

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Service telephone +86-10-57815020
Service email custserv@transgenbiotech.com